One bout of vibration exercise with vascular occlusion activates satellite cells

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Abstract: INTRODUCTION We tested the hypothesis that whole body vibration (WBV) is insufficient to expand satellite cell (SC) numbers 24 h post exercise, whereas WBV in combination with blood flow restriction (BFR) is. METHODS Twenty-five young men were randomly assigned to one of three groups: WBV, BFR exercise or WBVBFR. SC numbers were determined from muscle biopsies of the vastus lateralis using immunohistochemistry. RESULTS SC quantity and frequency (+99.38%, P = 0.012 and +77.1%, P = 0.010, respectively) only increased in the WBVBFR group. Similar results were obtained for the quantity and frequency of myogenin+ myonuclei (+139.0%, P < 0.001 and +148.4%, P < 0.001, respectively). CONCLUSION We conclude that modification of WBV by superimposing BFR induced activation and differentiation of SC in young men, all of which had not been observed with WBV or BFR alone. These data suggest that WBVBFR might represent a novel viable anabolic stimulus.

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One bout of vibration exercise with vascular occlusion activates satellite cells

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- Acute skeletal muscle satellite cell (SC) activation is associated with skeletal muscle hypertrophy. Although SC quantity has been reported to increase following a single bout of resistance exercise, data on muscle fibre type-specific SC quantity and/or activation status after single bout of vibration and/or occlusion exercise are presently lacking.

- By determining SCs from muscle biopsies of the *M. vastus lateralis* using immunohistochemistry, we conclude that modification of vibration exercise by superimposing occlusion induced activation and differentiation of SC in young men, all of which had not been observed with WBV or BFR alone.

Abstract

Introduction: We tested the hypothesis that whole body vibration (WBV) is insufficient to expand satellite cell (SC) numbers 24 h post exercise, whereas WBV in combination with blood flow restriction (BFR) is. Methods: Twenty-five young men were randomly assigned to one of three groups: WBV, BFR exercise or WBVBFR. SC numbers were determined from muscle biopsies of the vastus lateralis using immunohistochemistry. Results: SC quantity and frequency (+99.38%, P = 0.012 and +77.1%, P = 0.010, respectively) only increased in the WBVBFR group. Similar results were obtained for the quantity and frequency of myogenin+ myonuclei (+139.0%, P < 0.001 and +148.4%, P < 0.001, respectively). Conclusion: We conclude that modification of WBV by superimposing BFR induced activation and differentiation of SC in young men, all of which had not been observed with WBV or BFR alone. These data suggest that WBVBFR might represent a novel viable anabolic stimulus.

Key words: satellite cells, hypertrophy, exercise, whole body vibration, blood flow restriction

Abbreviations:

BFR, blood flow restriction; CSA, cross-sectional area; DNA, deoxyribonucleic acid; DAPI, 4',6-diamidino-2-phenylindole; HGF, hepatocyte growth factor; MND, myonuclear domain; MRF, muscle regulatory factor; MyHC, myosin heavy chain isoform; NGS, normal goat serum; NCAM, neural cell adhesion molecule; NO, nitric oxide; Pax7, transcription factor paired box protein 7; ROI, region of interest; SC, satellite cell; SD, standard deviation; VEGF, vascular endothelial growth factors; WBV, whole body vibration; 1RM, one repetition maximum.

Introduction

Whole body vibration (WBV) has long been suggested as an alternative training modality. However, despite some evidence that a single bout of WBV can cause a transient potentiation of muscle power, it remains inconclusive whether multiple bouts of WBV over several days and weeks can lead to sustained neuromuscular adaptations with longer-lasting increases in muscle power and force. In fact, some studies found that longer-term WBV (vibration frequency: 25–40 Hz, amplitude: 2.5–3.4 mm) can cause gains in knee extensor torque, countermovement jump power, and jumping height in young healthy individuals (Delecluse *et al.* 2003; Torvinen *et al.* 2003), comparable to those observed after resistance exercise, but others have not (Cochrane *et al.* 2004; Torvinen *et al.* 2002).

Where it was assessed, the increase in muscle power was paralleled by an increase in muscle mass (Bogaerts *et al.* 2007) or fat free mass (Roelants *et al.* 2004). However, these studies were conducted with elderly men and untrained women, respectively. For young healthy active individuals, there is no convincing evidence that long-term WBV can effectively increase muscle mass or muscle fibre cross-sectional areas (CSA). Accordingly, it has been argued that muscle hypertrophy is unlikely to occur after WBV in young healthy individuals (Pollock *et al.* 2010). The reason for this lack is that at the highest amplitude and frequencies of vibration, the greatest muscle activity is similar to that during fast walking (~1.7 m s^{-1}; Masumoto *et al.* 2004) and does not exceed 55% of isometric maximal voluntary contraction when typical protocols are applied (Pollock *et al.* 2010).

Skeletal muscle fibre hypertrophy is defined as an increase in muscle fibre volume, with or without the addition of myonuclei. This process is primarily driven by the accretion of myofibrillar proteins, which are arranged into sarcomeres that are aligned in parallel (defining fibre CSA) and in series (defining fibre length). The cellular mechanisms underlying myofibre hypertrophy include increases in translational efficiency and/or translational capacity. Furthermore, myofibre hypertrophy largely

depends on transcriptional capacity, which in turn results from increases in myofibre DNA content through the addition of myonuclei. Therefore, the number of myonuclei is a critical determinant of protein synthesis capacity by supplying the amount of DNA which is necessary to sustain gene transcription (Favier *et al.* 2008).

Within the multinucleated skeletal muscle fibre, each myonucleus regulates gene transcription and subsequent protein synthesis over a finite volume of cytoplasm (Cheek, 1985; O'Connor *et al.* 2007). This volume per nucleus, commonly named "myonuclear domain" (MND), is not a stable quantity as demonstrated by the fact that initial myofibre hypertrophy can expand the cytoplasmic volume associated with each myonucleus (Petrella *et al.* 2006). Nevertheless, a ceiling size on the MND has been suggested (Petrella *et al.* 2006) based on the concept that sufficient expansion of the MND possibly puts each nucleus under greater strain to supply the necessary gene products, driving a demand for the addition of new myonuclei to permit growth beyond the ceiling size (Petrella *et al.* 2008). Therefore, the recruitment of additional myonuclei may occur at later times after the initial hypertrophic stimulus or once existing MNDs have been maximized by protein accretion in response to muscle loading (Petrella *et al.* 2008).

The addition of myonuclei is facilitated by a pool of normally quiescent satellite cells (SC), which can be induced to proliferate, differentiate, and fuse as nuclear contributors to existing myofibres supporting growth and regeneration (Petrella *et al.* 2008). These cells are architecturally positioned below the basal lamina of the myofibre and are activated by a number of signals, including local and systemic growth factors (Hawke & Garry, 2001) and mechanical load (Tidball, 2005). Thus, activation, proliferation and differentiation of SC following exercise set the stage for robust myofibre hypertrophy following fusion, and act as markers for the anabolic potency of the training stimulus in

terms of hypertrophy (Bellamy *et al.* 2014).

In this study, we hypothesized that WBV is ineffective in stimulating SC in healthy young active men. Given that WBV leads to intensities comparable to those during low-intensity exercise and that low-intensity exercise performed under blood flow restricted (BFR) (ischaemic) conditions can induce hypertrophy (Abe *et al.* 2006; Ohta *et al.* 2003; Takarada *et al.* 2000) we further hypothesized that WBV under BFR conditions (WBVBFR) would increase the number of SC to a larger extent compared to WBV. To test our hypotheses, three groups of young active men performed either a static half-squat standing on a side-alternating vibration plate (WBV group), a static half-squat under BFR conditions without vibration (BFR group), or a static half-squat standing on a side-alternating vibration plate under BFR conditions (WBVBFR group). Before and 24 h after the stimulus, muscle biopsies were taken from the *vastus lateralis* muscle to analyse the SC response to exercise.

Methods

Participants

The study was approved by the ethics committee of the canton Zurich (Switzerland) and was conducted in accordance with the Declaration of Helsinki. Twenty-five recreationally active males volunteered to participate in this study. Participants were assigned to one of three intervention groups (either WBV, BFR or WBVBFR), matched for anthropological constitution (Table 1). They were involved in team or individual sports and performed 2-3 exercise sessions per week, of which at least one training sessions involved structured resistance exercise. All participants had been engaged in these activities on a regular basis for at least one year prior to the study. The resulting number of participants was $n = 8$, $n = 9$ and $n = 8$ for the WBV, BFR, and WBVBFR group, respectively.

Participants were fully informed about the purposes, benefits and risks associated with this intervention and completed a routine health questionnaire before giving written informed consent to their participation in this study.

Experimental procedures

The study consisted of baseline measurements of anthropological variables, a baseline muscle biopsy, one acute intervention (consisting of either a static half-squat with WBV, a static half-squat with BFR, or a static half-squat with WBVBFR), and a biopsy 24 h after the intervention.

Acute training intervention

All participants were asked to support a static half-squat position in a flexed knee angle of 135° (180°: knees fully extended). The knee angle was consistently checked using a manual goniometer. The feet were placed to the width of the pelvis and remained parallel. The WBV group performed the static half-squat exercise standing on a Galileo® vibration plate (Novotec, Pforzheim, Germany) oscillating at 30 Hz with an amplitude of 2.56 ± 0.42 mm. In the BFR group, tourniquet cuffs (0.09 m width, 076 m length; VBM, Sulz a.N., Germany) were affixed to the inguinal fold region of the participant's thigh and inflated to 200 mmHg (26.7 kPa) while performing squat exercise. Participants in the WBVBFR group performed the static half-squat exercise using WBV and BFR. For all groups, one duty cycle consisted of 4 min static half-squat and 3 min resting. This duty cycle was repeated three times. In the resting period, the cuffs were deflated to 100 mmHg (13.3 kPa) for 1 min and then deflated to 0 mmHg for the remaining time (BFR and WBVBFR groups only).

Muscle biopsy sampling

Two biopsies of the *M. vastus lateralis* were obtained from the dominant leg after local anesthesia with 1 % lidocaine from the middle portion of the dominant vastus lateralis muscle, using a 6-mm Bergström needle (Dixons Surgical Instruments, Essex, UK), with suction applied. The first biopsy served to generate baseline measures for variables of interest, including muscle fibre type distribution, fibre CSA, SC quantity and subsarcolemmal myonuclear number. The first biopsy was obtained 14–21 d before the training intervention in order to minimize any effects of the biopsy procedure on SC counts performed on the second biopsy (Dreyer *et al.* 2006). The second biopsy was taken 24 h after a single bout of WBV, BFR or WBVBFR exercise. All participants were instructed to refrain from exercise for 24 h before each biopsy. Biopsies were obtained from the mid-portion of the *M. vastus lateralis* muscle almost 18 cm proximal to the patella, approximating the midline of the quadriceps muscle group. The second biopsy was performed at a distance of 1-2 cm proximal or distal (randomly assigned) to the baseline biopsy site. Generally 100-150 mg of muscle tissue was obtained from each biopsy.

Muscle biopsy analyses

Transverse consecutive serial sections (8-10 µm) of the embedded muscle biopsy specimen were cut at −22 °C using a cryostat (Leica CM 3050 S, Solms, Germany) and were mounted on Fisherbrand Superfrost/Plus microscope slides (Fisher Scientific, Pittsburgh, PA, USA). The serial cryocut cross-sections were stained using the myofibrillar adenosintriphosphatase (mATPase) method at pH 4.6 as previously described (Mueller *et al.* 2014). Muscle fibres were classified according to their myosin heavy chains (MyHC) isoform into MyHC-1, MyHC-2A and MyHC-2X. For the fibre type specific determination of the CSA, MND and SC assignments, a method used for myofibre typing based on MyHC isoform immunoreactivity was applied (Kim *et al*. 2005). Specifically, monoclonal antibodies against MyHC specific to human for MyHC-1 (mouse MAb NCL-MHCs, Novacastra Laboratories,

Newcastle upon Tyne, UK, 1:100), MyHC-2A (anti-MHC 2a mouse MAb, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA, USA, 1:80) and for the cell borders (anti-laminin mouse MAb, Novacastra Laboratories, Newcastle upon Tyne, UK, 1:80) were used. As second antibodies, DyLights 488 and 594 [goat anti-mouse IgG (H+L), DyLight 488/594, Pierce Antibody Products, Waltham, MA, USA, 1:200] were applied as described by Kim *et al.* 2005. SC and differentiation status were identified on consecutive 8 µm thick cross-sections by immunohistochemical staining's for the paired box transcription factor Pax7 (Walker *et al.* 2012) and the muscle regulatory factor (MRF) myogenin (Hanssen *et al.* 2013). Specifically, sections were fixed using a 4% paraformaldehyde solution for 5 min at room temperature followed by 3 times 5 min rinses with phosphate buffered saline (PBS) solution. Sections were blocked with 10% normal goat serum (NGS) plus 0.5% Triton-X 100 for 45 min. Sections were incubated overnight at 4 °C with primary antibodies, mouse anti-Pax7 (Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA, USA, 1:300) or rabbit anti-myogenin (Santa Cruz Biotechnology, Dallas, TX, USA, 1:200). Anti-laminin mouse MAb (Novacastra Laboratories, Newcastle upon Tyne, UK, 1:80) was applied at 37 °C in the dark for 30 min in order to detect cell borders. Sections were rinsed 3 times for 5 min each with PBS and blocked with 5% NGS in PBS, followed by 30 min incubation with secondary antibodies, DyLight 594 goat anti-rabbit and goat anti-mouse [goat anti-mouse IgG (H+L), DyLight 594, Pierce Antibody Products, Waltham, MA, USA, 1:200] and DyLight 488 goat anti-mouse [goat anti-mouse IgG (H+L), DyLight 488, Pierce Antibody Products, Waltham, MA, USA, 1:200], at 37 °C in the dark. Sections were rinsed 3 times for 5 min each with PBS and mounted with cover slips in mounting medium (ProLong Gold Antifade Reagents with DAPI, Molecular Probes, Invitrogen, Carlsbad, CA, USA).

Image analyses

For each muscle sample, non-overlapping images were randomly chosen. The staining protocol resulted in myonuclei staining blue, MyHC-1 red, MyHC-2A and laminin green, while MyHC-2X remained uncoloured (black; Fig. 1.1 a; Fig. 1.2 a). Myofibres positive for MyHC-1 and negative for MyHC-2 were classified as type 1, fibres positive for MyHC-2A and negative for MyHC-1 were classified as type 2A, and fibres negative for both MyHC-1 and MyHC-2A were classified as type 2X. SC (Pax7+ cells) had to be localized to the membrane (laminin stained green), stained red and co-located with DAPI (blue; Fig. 1.1 b,c,d). The number of SC divided by the number of muscle fibres was categorized as SC quantity. The number of SC divided by the total number of subsarcolemmal myonuclei was categorized as SC frequency. Likewise, to assure myogenin fibre type specificity, the MyHC staining resulted the same as described above (Fig. 1.2 a). Myogenin positive myonuclei (myogenin+ myonuclei) were localized to the membrane (laminin stained green) of a muscle fibre and identified as any nuclei stained red co-located with DAPI (blue; Fig. 1.2 b,c,d). The number of myogenin+ cells divided by the number of muscle fibres was categorized as quantity of myogenin+ myonuclei. The number of myogenin+ cells divided by the total number of subsarcolemmal myonuclei was categorized as frequency of myogenin+ myonuclei. Results are shown for both MyHC isoforms taken together (*i.e.* all fibre type), MyHC-1 and MyHC-2. Muscle fibre type-specific association of the SC was conducted by assigning the randomly chosen area to the consecutive muscle cross-sections, immunostained to identify fibre types. As the correlation coefficient for counting SC continues to increase with increasing numbers of fibres in the analysis (Mackey *et al.* 2009), we analysed at least a total of 50 MyHC-1 and 75 MyHC-2 muscle fibres to guarantee reliability. Fibre CSA were calculated by fully encircling the laminin borders of the MyHC-stained cells of at least 50 fibres per MyHC isoform and computing the area within the borders (McCall *et al.* 1998). Within each image, every fibre cross-sectional area with its corresponding number of myonuclei per fibre was used to calculate the MND, which was defined as the ratio of each fibre cross-sectional area divided by the number of myonuclei per fibre, i.e. the area controlled by a single nucleus. We used the NIH Image J Software (version 1.44o, National Institutes of Health, Bethesda, MD, USA) and Photoshop

Pro CS6 (Adobe Systems Incorporated, San Jose, CA, USA) for all fibre analyses. Fibre circularity was calculated using the formula $(4\pi \times CSA)/(perimeter)^2$ and only fibres with a circularity higher than 0.65 were considered for analysis (perfect circle = 1.0). Due to insufficient number of MyHC-2X fibres, analyses of the variables were therefore confined to MyHC-1 and MyHC-2, with MyHC-2 variables being a weighted average based on the relative distributions of MyHC-2A and MyHC-2X myofibres. The same investigator carried out all analyses manually.

A total of 4048 (baseline-training) and 3862 (acute-training) fibres from 25 participants were analysed for SC quantity and SC frequency. A mean of 162 ± 5 fibres and an average of 11 ± 0.5 SC were analysed per biopsy sample. An average of 71 ± 13 fibres were analysed for the determination of CSA, myonuclear number and MND per biopsy sample. On average, 790 ± 260 muscle fibres per participant (baseline biopsy) were classified according to their MyHC isoform into MyHC-1 (389 ± 121), MyHC-2A (328 ± 158) and MyHC-2X (74 ± 114). The results for each variable are reported as general group means as well as mean per fibre type for each group.

Body composition

A densitometer (Lunar iDXA™, GE Healthcare, Madison, WI, USA) was used for the determination of thigh lean mass. The delineation in region of interest (ROI) for the legs was done manually using the integrated software (encore, GE Healthcare, Madison, WI, USA; version 11.40.004) as follows: ROI's upper boundary = horizontal line just below the ischium; ROI's lower boundary = horizontal line below the feet; ROI's lateral boundaries = outer leg cuts.

Statistical analysis

Data are presented as mean values ± standard deviations (SD). Normality of data was visually ascertained by Q–Q-plots. Baseline values between the training groups were analysed with a one-way analysis of variance. For the detection of significant differences between groups over time, a univariate general linear model was applied. For this analysis, the differences acute–baseline (%) of each variable was compared between the groups. Significant differences within groups from baseline-to acute-intervention were displayed by parameter estimates. This analysis tested the null hypothesis that Δ parameter was 0. If Δ parameter had a *P*-value lower than the level of significance, the null hypothesis was rejected meaning that Δ parameter was significantly different from 0. The level of significance was set at $P < 0.05$. All statistical analyses were performed using the software SPSS Statistics 20.0 (SPSS, Chicago, IL, USA).

Results

Muscle fibre cross-sectional area, myonuclear number and myonuclear domain

CSA did not differ between groups at baseline (Table 2). Likewise, the number of subsarcolemmal myonuclei was not different at baseline either (Table 2). Accordingly, no differences in the MND were observed at baseline between groups (Table 2).

Muscle fibre distribution

At baseline, MyHC-1 proportion was significantly higher in the BFR-group compared to the WBV-group ($P = 0.035$, Table 3). No further statistical differences were present for MyHC fibre distribution between intervention groups.

Baseline SC quantity and frequency

The SC quantity and frequency per all myofibre type was not different at baseline between groups (Fig. 2A, D). In addition, when expressed per fibre type, the SC quantity and frequency did not differ at baseline between groups, either (for SC quantity, Fig. 2B, E; for SC frequency, Fig. 2C, F).

Acute increases in SC quantity and frequency

The SC quantity per all myofibre remained unchanged in the WBV and BFR group (Fig. 2A), while it increased in the WBVBFR group (P = 0.002; Fig. 2A). Furthermore, when expressed per fibre type, SC quantity increased in the WBVBFR group only [P = 0.016 and P = 0.002, for MyHC-1 (Fig. 2B) and MyHC-2 (Fig. 2C), respectively]. There was no corresponding between-group difference in MyHC-fibre type-specific SC quantity (Fig. 2A). The SC frequency per all myofibre did not change in the WBV and BFR group (Fig. 2D). SC frequency per all myofibre increased in the WBVBFR group (P = 0.010; Fig. 2D). Fibre type specific SC frequency did not increase in any group (Fig. 2E, F).

Baseline quantity and frequency of myogenin+ myonuclei

The quantity and frequency of myogenin+ myonuclei per all myofibre was not different at baseline between groups (Fig. 3A, D). In addition, when expressed per fibre type, the quantity and frequency of myogenin+ myonuclei did not differ at baseline between groups, neither (Fig. 3B, C, E, F).

Acute increases in quantity and frequency of myogenin+ myonuclei

The quantity of myogenin+ myonuclei per all fibre type remained unchanged in the WBV and BFR group (Fig. 3A). Therefore, the quantity of myogenin+ myonuclei per all fibre type did not change in these groups. By contrast, the quantity of myogenin+ myonuclei per all fibre type increased in the WBVBFR group ($P < 0.001$; Fig 3A). The quantity of myogenin+ myonuclei per all fibre type was different between groups ($P = 0.011$ for WBVBFR *vs.* WBV and $P = 0.006$ for WBVBFR *vs.* BFR; Fig. 3A). Furthermore, when expressed per fibre type, the quantity of myogenin+ myonuclei increased in the WBVBFR group only [$P < 0.001$ and $P < 0.001$, for MyHC-1 (Fig. 3B) and MyHC-2 (Fig. 3C), respectively]. The frequency of myogenin+ myonuclei per all fibre type in the WBV and BFR group did not change (Fig 3D). The frequency of myogenin+ myonuclei per all fibre type increased in the WBVBFR group only ($P < 0.001$; Fig. 3D). The increase in quantity of myogenin+ myonuclei per all fibre type was different between groups ($P = 0.048$ for WBVBFR *vs.* WBV; Fig. 3D). In the WBVBFR and BFR group, the frequency of myogenin+ myonuclei increased for MyHC-1 and MyHC-2 (MyHC-1: $P = 0.013$ and $P = 0.012$ for WBVBFR and BFR, respectively; Fig 3E; MyHC-2: $P = 0.012$ and $P = 0.039$ for WBVBFR and BFR, respectively, Fig. 3F). No changes were observed for the WBV group (Fig. 3E, F).

Discussion

This study provided evidence that a single bout of WBV exercise did affect neither SC quantity nor the number of myogenin+ myonuclei 24 h post exercise in recreationally trained young men. Similarly, BFR exercise did not elicit either activation or differentiation of SC. In contrast, SC quantity was increased 24 h after a single bout of WBVBFR exercise and a concomitant increase in the number of myogenin+ myonuclei occurred.

The MND, which was below the proposed ceiling size of approximately 2000 μm^2 (Petrella *et al.*

2006) was not different between groups at baseline. Consequently, it is unlikely that the facilitated increase in SC quantity, which occurred exclusively in the WBVBFR group, was attributed to baseline differences in the potential to expand the MND. Notably, no between-group differences in SC quantity were observed, despite between-group differences in muscle fibre type distribution. In fact, the proportion of MyHC-1 fibres was higher for BFR than WBV at baseline, yet SC quantity was similar. Our data do not support the notion that MyHC-1 fibres have more SC than MyHC-2 fibres but instead lend further credence to the results of Kadi *et al.* 2006, which concluded that SC distribution in human *vastus lateralis* is not dependent on fibre type composition.

It can also be excluded beyond reasonable doubt that between-group differences in the myogenic potential dictated the observed between-group differences in SC proliferation and differentiation after acute exercise in this study. As shown by Petrella *et al.* 2008, high responders to resistance exercise-induced myofibre hypertrophy exhibited a significantly larger baseline SC quantity than moderate or low responders, as evidenced by the higher number of positive neural cell adhesion molecule cells (NCAM+) per fibre as well as the number of NCAM+ cells relative to total subsarcolemmal myonuclear number in this group. Thus, one's myogenic potential might at least partially be predetermined by the availability of SC before training (Petrella *et al.* 2006). Given that no difference in SC quantity was observed between groups at baseline, all three groups started with the same myogenic potential in terms of having the same availability of SC at baseline. The absence of between-group differences in baseline SC quantity in this study supports the notion that the SC quantity in recreationally trained muscle is tightly regulated and thus, held fairly constant in steady-state conditions (Dhawan & Rando, 2005). Similar to SC quantity, the number of differentiating SC (myogenin+ SC) was equal for all three groups at baseline, too. As no group differences were found at baseline for this marker of differentiation, it is unlikely that group differences in cell cycle progression could explain the group difference in the response to acute exercise.

This is the first study to show that stand-alone, neither WBV nor BFR were sufficient to induce proliferation and differentiation of SC 24 h post intervention, while the simultaneous combination of the two stimuli (WBVBFR) was. A substantial increase in SC quantity and a concomitant rise in the number of myogenin+ myonuclei occurred 24 h after a single bout of WBVBFR exercise in a non muscle fibre type-specific manner. Several studies have shown that SC numbers are elevated as soon as 24 h after exercise, especially for resistance exercise-type regimen involving lengthening muscle actions (Cermak *et al.* 2013; O'Reilly *et al.* 2008). In particular, and similar to the results obtained for WBVBFR, it was shown that resistance exercise induced a significant increase in the number of Pax7+ cells 24 h post exercise in young men (Walker *et al.* 2012). The effect of WBVBFR on the number of Pax7+ cells (+99.5%) was similar to the one observed for resistance exercise (+147.6%). Our finding is intriguing, since the development of a low-mechanical strain intervention to increase muscle function has considerable clinical significance. There are many conditions where traditional high force paradigms for muscle adaptation cannot be used. For example, in injured and postsurgical patients with compromised musculotendinous integrity, high force loading of a joint or muscle is frequently contraindicated. In this regard, WBVBFR could represent an interesting alternative.

As expected, the WBV group did not show any elevations in SC quantity and frequency, possible reason being that the applied vibration mode (vibration type, amplitude and frequency) did not elicit enough metabolic fatigue and/or mechanical strain for activating anabolic-signalling pathways in young, healthy trained men. As demonstrated by Wahl *et al.* 2014, high-intensity training (*i.e.* 4x4 min or 4x30 s high-intensity endurance exercise) stimulated a transient increase in circulating levels of vascular endothelial growth factors (VEGF) and hepatocyte growth factors (HGF), whereas high-volume training (*i.e.* 120 min at 55% peak power output) had no influence on VEGF and HGF. This points towards an intensity dependence in cell cycle transition of differentiated SCs, which was not

accomplished by WBV alone. Despite that WBV has been reported to increase electromyographic activity (Perchthaler *et al.* 2013; Pollock *et al.* 2012; Yasuda *et al.* 2008), motor unit recruitment (Pollock *et al.* 2012; Yasuda *et al.* 2008; Manini *et al.* 2011), static half-squat on a vibrating platform did not induce up regulation in the numbers of myogenin+ myonuclei and therefore do not promote myotube fusion in young, healthy trained men. Equally, 24 h after a single bout of BFR exercise, neither SC quantity nor the number of myogenin+ myonuclei were changed. In contrast to the results of Wernbom *et al.* 2013, which measured an about three- to fourfold elevation of differentiated SC, our data showed no elevated number of myogenin+ myonuclei after a single bout of BFR. Thus, only a single bout of BFR exercise combined with dynamic resistance exercise could cause elevations in SC numbers. It may be concluded, that just standing in the half-squat position with BFR, did no elicit activation and differentiation of SC.

In conclusion, this study has provided evidence that modification of WBV by superimposing vascular occlusion induced activation and differentiation of SC, all of which had not been observed with WBV or BFR alone. The increase in SC activation and differentiation status was not fibre-type specific, indicating that superimposing WBV with BFR may change motor unit recruitment pattern and fibre activation, thereby providing the basis for a new anabolic treatment in recreationally young trained men. Based on these findings, we recommend that trained young men who aim at further improving their anabolic potential should also consider WBVBFR. Finally, WBVBFR could also represent an interesting anabolic alternative to high force exercise in patients and/or elderly individuals.

Additional information

Competing interests

None declared.

Author contributions

Experiments were performed in the laboratories of M.T. and H.J. D.A., S.M, U.B., M.F., M.T. were involved in the conception and design of the experiments. D.A., S.M., M.A., H.J., M.T. were involved in the acquisition, analysis or interpretation of the data collection. All authors were involved in the analysis and interpretation of data, and in drafting the article and revising it critically for important intellectual content. All authors approved the final version of this manuscript. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Tables

Table 1 Physical characteristics

	WBV	BFR	WBVBFR
Age (years)	26.8 ± 1.2	27.7 ± 4.6	26.6 ± 4.0
Height (cm)	176.6 ± 5.9	180.9 ± 9.1	183.1 ± 6.9
Body mass (kg)	78.9 ± 14.0	83.2 ± 10.0	79.2 ± 10.0
Leg lean mass (kg)	20.9 ± 3.5	22.1 ± 2.5	22.8 ± 2.5

Values are means ± SD for three groups of young active men performing either a static half-squat standing on a side-alternating vibration plate (WBV: n = 8), a static half-squat under blood flow restriction conditions without vibration (BFR: n = 9), or a static half-squat standing on a side-alternating vibration plate under BFR conditions (WBVBFR: n = 8).

Table 2 Muscle fibre characteristics and myonuclear domain.

	Group	MyHC	Baseline	Acute
Muscle fibre CSA [μm^2]	WBV	All	4970.9 ± 962.4	4986.8 ± 1173.1
		MyHC-1	4818.6 ± 1048.5	4736.3 ± 1128.2
		MyHC-2	5123.2 ± 1121.9	5237.3 ± 1328.8
	BFR	All	5777.2 ± 1467.7	6004.6 ± 2021.8
		MyHC-1	5461.5± 1372.9	5500.7 ± 2031.9
		MyHC-2	6092.9 ± 1769.0	6508.5 ± 2149.4
	WBVBFR	All	5041.9 ± 529.1	5268.9 ± 792.5
		MyHC-1	4549.6 ± 493.8	4771.5 ± 940.1
		MyHC-2	6092.9 ± 1769.0	6508.5 ± 2149.4
Subsarcolemmal myonuclei/Fibre	WBV	All	3.2 ± 0.4	3.3 ± 0.4
		MyHC-1	3.4 ± 0.5	3.5 ± 0.5
		MyHC-2	3.0 ± 0.4	3.1 ± 0.4
	BFR	All	3.4 ± 0.4	3.5 ±0.5
		MyHC-1	3.4 ± 0.3	3.7 ± 0.5
		MyHC-2	3.2 ± 0.5	3.3 ± 0.6
	WBVBFR	All	3.3 ± 0.4	3.4 ± 0.3
		MyHC-1	3.4 ± 0.4	3.6 ± 0.4
		MyHC-2	3.1 ± 0.4	3.2 ± 0.2
MND [μm^2]	WBV	All	1772.6 ± 524.4	1664.3 ± 347.8

	MyHC-1	1602.1 ± 455.4	1434.6 ± 336.5
	MyHC-2	1943.0 ± 632.1	1893.9 ± 414 .7
BFR	All	2004.9 ± 573.8	1974.6 ± 638.1
	MyHC-1	1784.8 ± 517.6	1710.8 ± 609.3
	MyHC-2	2224.9 ± 713.9	2238.4 ± 686.9
WBVBFR	All	1821.9 ± 279.3	1845.9 ± 474.4
	MyHC-1	1553.9 ± 271.5	1517.9 ± 414.5
	MyHC-2	2089.9 ± 322.1	2173.9 ± 605.3

Mean values of cross-sectional area (CSA), subsarcolemmal myonuclei per fibre and myonuclear domain (MND) for all myosin heavy chains (MyHC) isoforms MyHC-1 and MyHC-2, before (baseline) and 24 h after (acute) the exercise intervention for the three groups of young active men performing either a static half-squat standing on a side-alternating vibration plate (WBV: n = 8), a static half-squat under blood flow restriction conditions without vibration (BFR: n = 9), or a static half-squat standing on a side-alternating vibration plate under BFR conditions (WBVBFR n = 8). Values are means ± SD.

Table 3 Muscle fibre type distribution

	WBV	BFR	WBVBFR
MyHC-1 [%]	42.8 ± 11.2	55.3 ± 8.8 *	53.7 ± 7.8
MyHC-2A [%]	43.2 ± 12.8	39.9 ± 9.3	39.2 ± 8.3
MyHC-2X [%]	14.0 ± 18.3	4..8 ± 3.6	7.1 ± 5.8

Myosin heavy chains (MyHC) isoform distribution of the three groups of young active men performing either a static half-squat standing on a side-alternating vibration plate (WBV: n = 8), a static half-squat under blood flow restriction conditions without vibration (BFR: n = 9), or a static half-squat standing on a side-alternating vibration plate under BFR conditions (WBVBFR: n = 8). Values are means ± SD, * P < 0.05, significant differences relative to WBV group.

Captions

Figure 1.1) Representation of fibre type-specific analyses of skeletal muscle satellite cell (SC) quantity for the WBV group (A), BFR group (B) and WBVBFR (C) group, respectively.

a) Representative image of myosin heavy chains (MyHC) isoform. MyHC-1 (red), MyHC-2A (green), MyCH-2X (black), subsarcolemmal myonuclei (blue) and cell borders (green), b) Higher magnification of selected area (white box), co-localization of SC (red) and subsarcolemmal myonuclei (blue) with cell border lines (green), c) Co-localization of SC (red) and subsarcolemmal myonuclei (blue), d) SC (red), Scale bar represents 100 μm (a). Scale bar represents 20 μm (b,c,d).

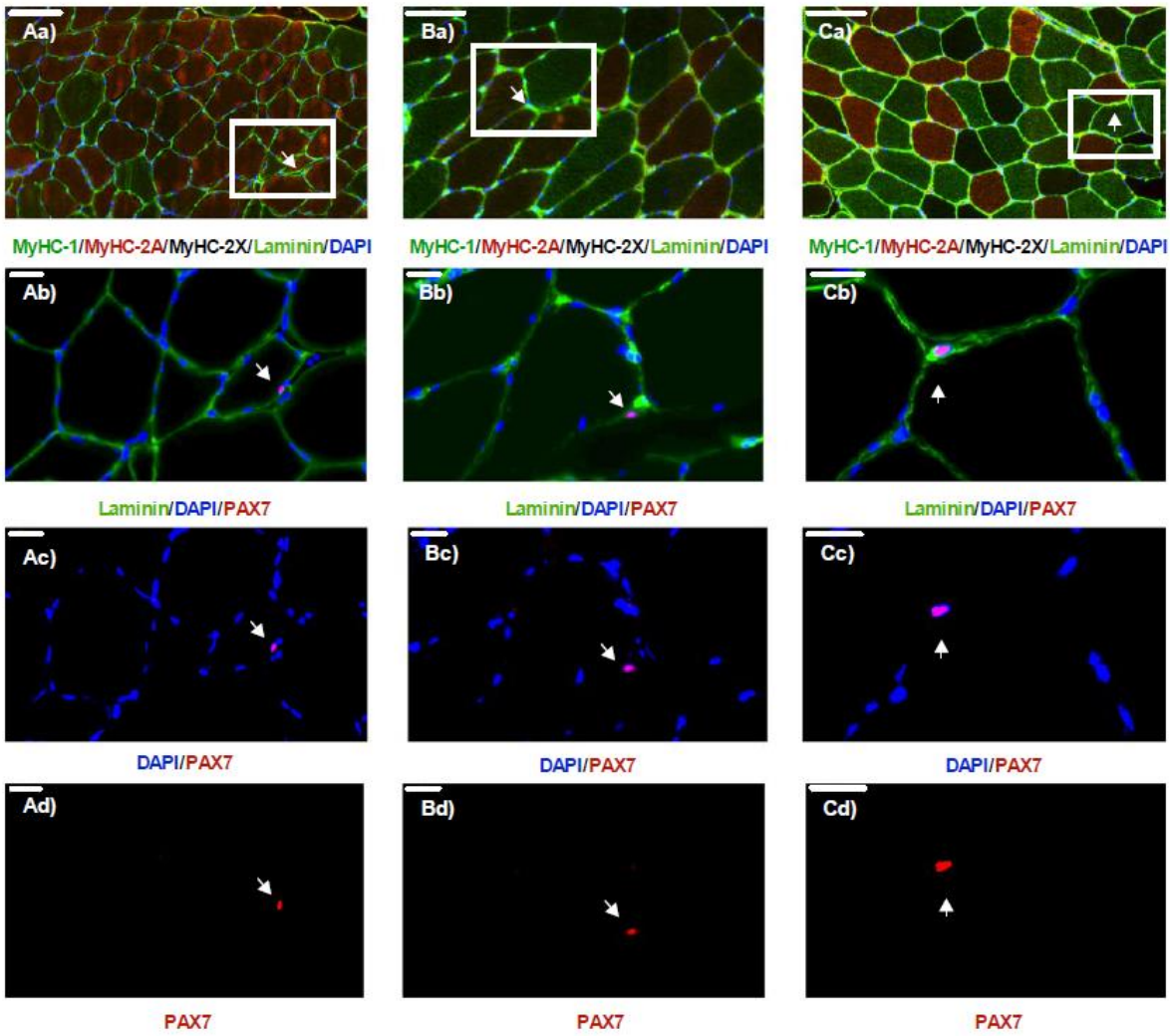

Figure 1.2) Representation of fibre type-specific analyses of skeletal muscle myogenin+ myonuclei quantity for the WBV group (A), BFR group (B) and WBVBFR (C) group, respectively.

a) Representative image of myosin heavy chains (MyHC) isoform. MyHC-1 (red), MyHC-2A (green), MyCH-2X (black), subsarcolemmal myonuclei (blue) and cell borders (green), b) Higher magnification of selected area (white box), co-localization of SC (red) and subsarcolemmal myonuclei (blue) with cell border lines (green), c) Co-localization of myogenin+ myonuclei (red) and subsarcolemmal myonuclei (blue), d) Myogenin+ myonuclei (red). Scale bar represents 100 μm (a). Scale bar represents 20 μm (b,c,d).

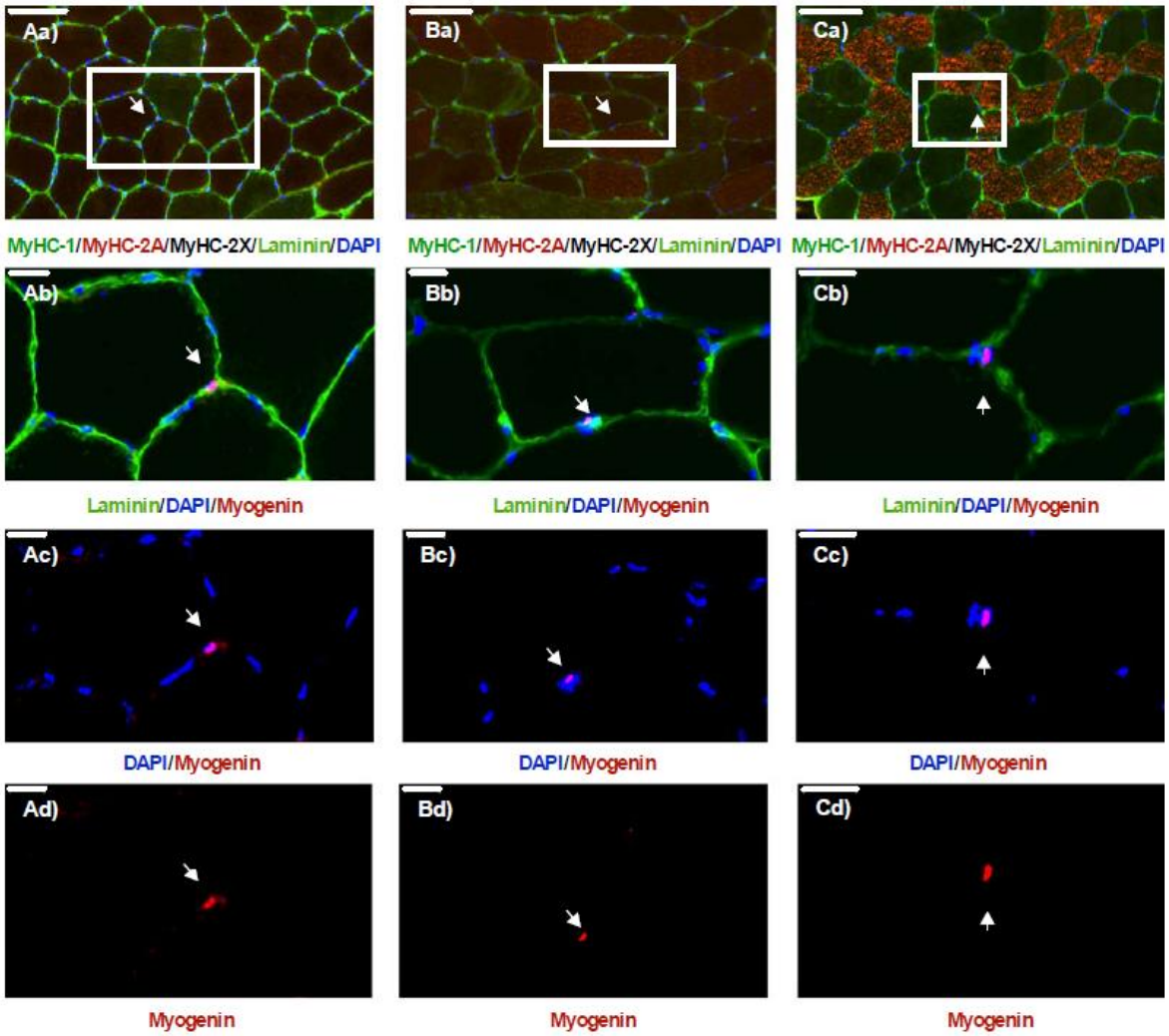

Figure 2: A) Total (*i.e.* all fibre type) satellite cell (SC) quantity from the *vastus lateralis* muscle of young men before (baseline: white bars) and 24 h after (acute: black bars) performing either a static half-squat standing on a Galileo side-alternating vibration plate (WBV: n = 8), a static half-squat under blood flow restriction (BFR) conditions without vibration (BFR: n = 9), or a static half-squat standing on a Galileo side-alternating vibration plate under BFR (WBVBFR: n = 8), B) SC quantity for MyHC-1, C) SC quantity for MyHC-2, d) Total (*i.e.* all fibre type) satellite cell (SC) frequency, e) SC frequency for MyHC-1, f) SC frequency for MyHC-2. Bars and error bars represent mean values and SD. *P < 0.01, significant differences within group baseline vs. acute.

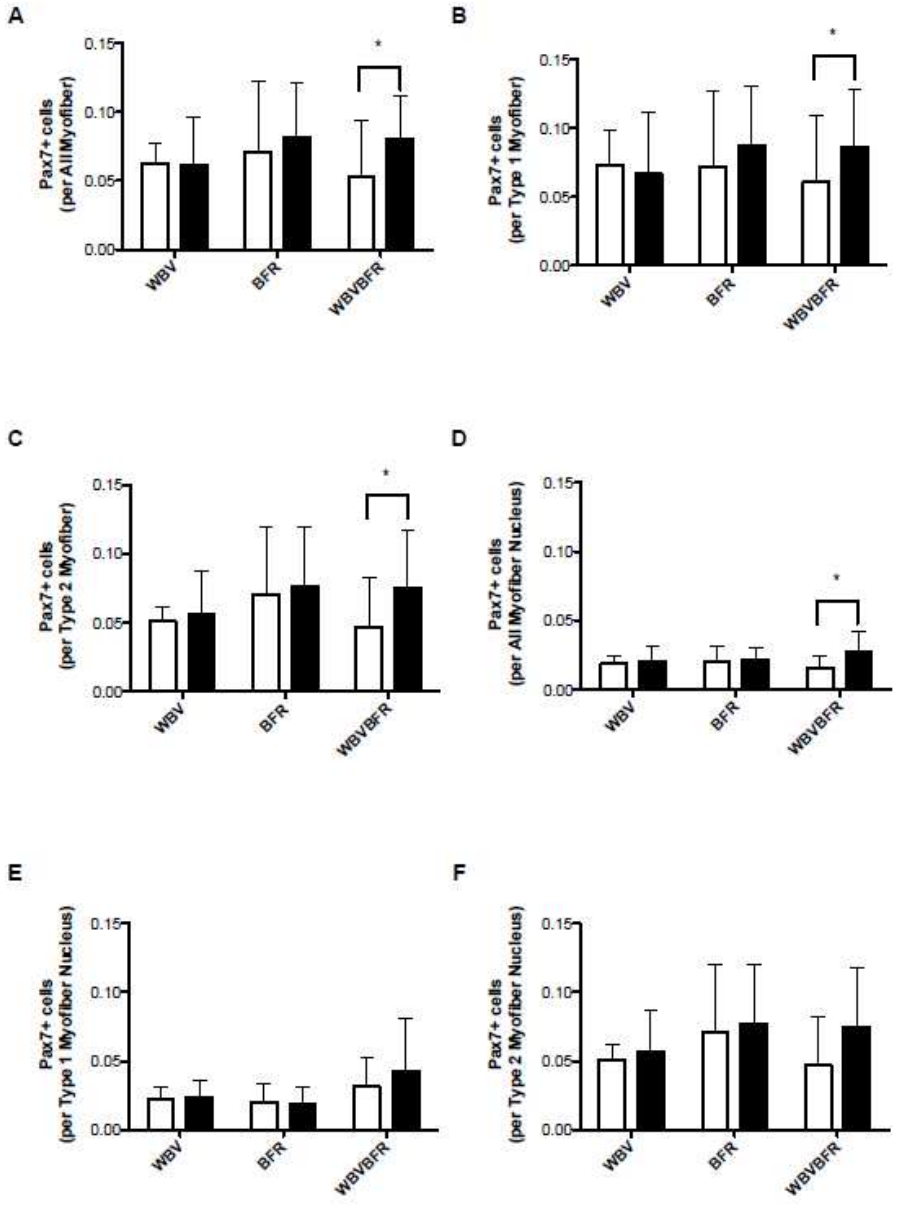

Figure 3: A) Total (*i.e.* all fibre type) quantity of myogenin+ myonuclei from the *vastus lateralis* muscle of young men before (baseline: white bars) and 24 h after (acute: black bars) performing either a static half-squat standing on a Galileo side-alternating vibration plate (WBV: n = 8), a static half-squat under blood flow restriction (BFR) conditions without vibration (BFR: n = 9), or a static half-squat standing on a Galileo side-alternating vibration plate under BFR (WBVBFR: n = 8). B) Myogenin+ myonuclei quantity for MyHC-1, C) Myogenin+ myonuclei quantity for MyHC-2, D) Total (*i.e.* all fibre type) frequency of myogenin+ myonuclei E) Myogenin+ myonuclei frequency for MyHC-1, F) Myogenin+ myonuclei frequency for MyHC-2. Bars and error bars represent mean values and SD. * P <0.05, *** P <0.001, significant differences within group baseline vs. acute. # P <0.05, ## P < 0.01, significant differences between groups baseline vs. acute.